Mater, a maternal effect gene required for early embryonic development in mice

Maternal effect genes produce mRNA or proteins that accumulate in the egg during oogenesis. We show here that Mater, a mouse oocyte protein dependent on the maternal genome, is essential for embryonic development beyond the two-cell stage. Females lacking the maternal effect gene *Mater* are sterile. Null males are fertile.

Maternal products control the developmental program until embryonic genome activation takes place. Maternal effect genes that are important in early embryonic development have been well documented in *Drosophila melanogaster* and *Xenopus laevis*[1,2], but their presence has only been inferred in mammals[3]. In mice, embryonic transcription is first detected in the late one-cell zygote stage and is required for development beyond the two-cell stage[4–6]. The factors governing this transition from the maternal to the embryonic genome are unknown.

We previously identified Mater as an oocyte antigen in a mouse model of autoimmune premature ovarian failure[7]. *Mater* is a single-copy gene expressed exclusively in oocytes[7,8]. *Mater* transcripts accumulated during oogenesis, but were not detected in early embryos. Mater protein was first detected in the cytoplasm of growing oocytes and remained present through the late blastocyst stage (data not shown). To determine its function, we generated *Mater*-null mouse lines in which homozygous females did not express either Mater transcripts or protein. *Mater*-null mice were born in the expected mendelian ratios with equal sex distribution. No phenotypic abnormalities were observed from birth through adulthood.

The *Mater*-null ovaries had a normal complement of primordial follicles and all stages of follicular development were represented (data not shown). Corpora lutea, indicating past spontaneous ovulation, were also present. The *Mater*-null females ovulated normally in response to stimulation with exogenous gonadotropins. Sexually mature *Mater*-null females had regular 5.67±0.22 day oestrus cycles and their mating behaviours seemed normal. The mice produced no litters, however, even after five months of mating with normal males, whereas the homozygous null males and heterozygous females had normal fertility.

Ova lacking Mater were fertilized normally *in vivo* (Fig. 1). The number and morphology of zygotes and two-cell embryos from the *Mater*-null females were similar to those from normal females. By three or four days after mating, however, the embryos from *Mater*-null females still remained at the two-cell stage or had begun to degenerate. Thus, fertilization is normal in *Mater*-null females and the resulting zygotes can progress through the first cleavage, but subsequent development is arrested at the two-cell stage. We conclude that an arrest of early embryogenesis accounts for the infertility in *Mater*-null females.

Inhibiting transcription with α-amanitin is also known to induce a block in embryonic development at the two-cell stage[4,5]. We evaluated transcription in null and control embryos using BrUTP incorporation[9,10]. *De novo* RNA transcription was decreased in the one- and two-cell embryos lacking Mater (Fig. 2*a–g*). Nevertheless, the two-cell embryos without Mater were able to synthesize TRC, the transcription-related complex dependent on embryonic genome activation in mice[11–13], although at a level 60% that of normal (Fig. 2*i*). This indicates that Mater is not critical for initiation of all transcription–translation machinery in early embryos. We saw progressive degradation of ribosomal RNA (Fig. 2*h*) and representative mRNAs in ova and early embryos lacking Mater.

Our results demonstrate that *Mater* is a novel maternal effect gene required for embryonic development beyond the two-cell stage in mice. The reduced embryonic transcription in the embryos from *Mater*-null mothers may result from abnormalities in transcription machinery, genomic DNA access or nuclear chromatin templates. Mater contains a leucine-rich domain and a short leucine zipper[8],

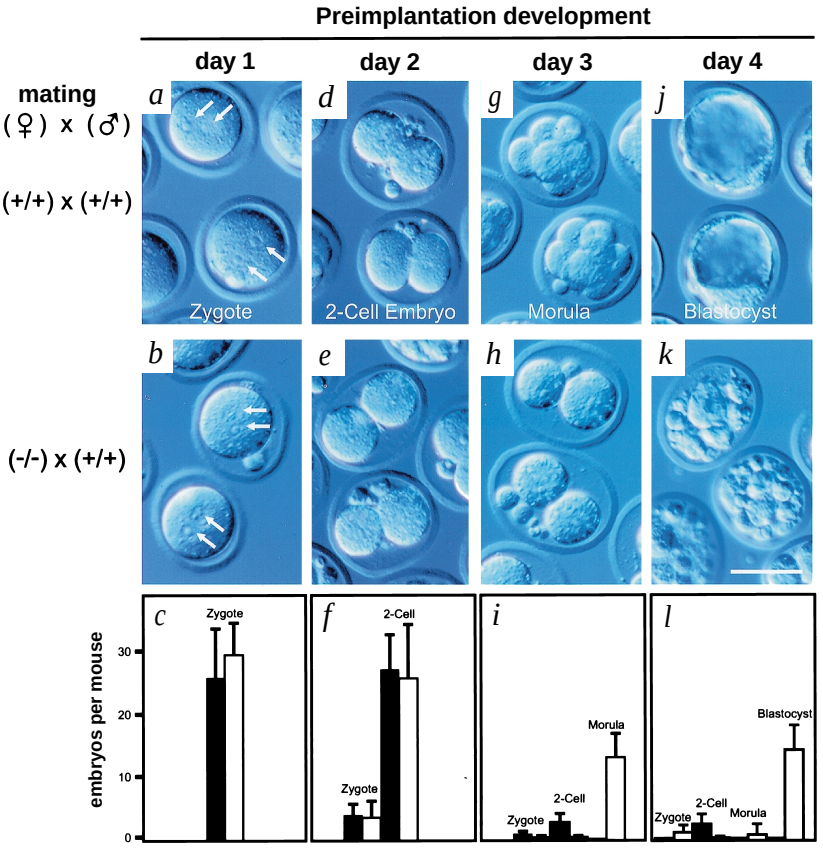

Fig. 1 *In vivo* development of embryos derived from *Mater*-null female mice. Embryos from normal (*a*,*d*,*g*,*j*) or *Mater*-null (*b*,*e*,*h*,*k*) females were isolated one (***a***,***b***,***c***), two (***d***,***e***,***f***), three (***g***,***h***,***i***) or four (***j***,***k***,***l***) days after human chorionic gonadotropin (hCG) and mating with normal males (+/+). The unfixed embryos were photographed using Nomarski optics. The arrows indicate pronuclei in one-cell zygotes. Scale bar, 50 μm. The bar graphs depict the average number of embryos from *Mater*-null (filled bar) and normal (open bar) females (*c*,*f*,*i*,*l*). Each bar represents the average of 4–5 experiments±s.e.m. The GenBank accession numbers for *Mater* are AH009243, AF143559–AF143573 and AF074018 (originally referred as to OP1).

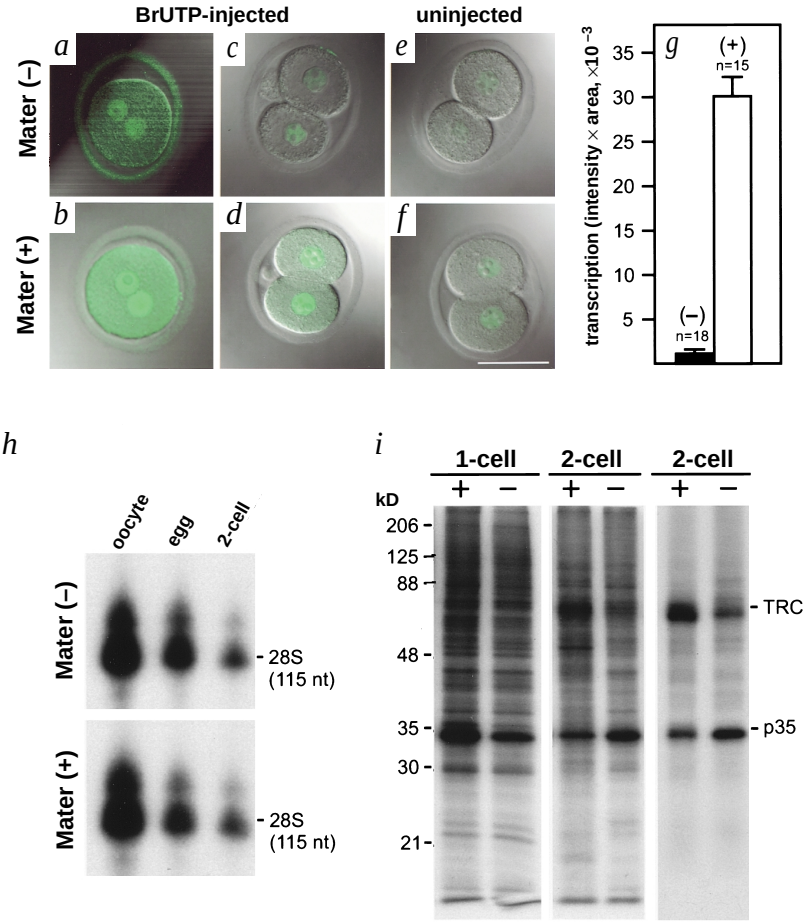

Fig. 2 *De novo* transcription and translation in embryos lacking Mater. BrUTP incorporation into the nucleus was measured in one- (***a***,***b***) and two-cell (***c***,***d***) embryos from *Mater*-null (*a*,*c*) and normal (*b*,*d*) females using laser-scanning confocal microscopy and anti-BrUTP antibody. ***g***, Incorporation of BrUTP was quantified in two-cell embryos with (+) and without (–) Mater using arbitrary units of fluorescence after subtraction of background in uninjected controls (***e***,***f***). Scale bar, 50 μm. ***h***, RNase protection assay. ^{32}P-labelled antisense probes (153 nt) protected 115-nt 28S rRNA from 50 growing oocytes, eggs and 2-cell embryos with (+) and without (–) Mater. ***i***, Fluorography of *de novo* protein synthesis in 1-cell zygotes (left) and 2-cell embryos (middle, right) with (+) or without (–) Mater. Each lane contained proteins from 10 embryos without extraction (left, middle) or with extraction of the TRC (65–75 kD) and p35 (right).

structural motifs for protein–protein interactions[14,15]. Identification of putative interacting proteins should provide insights into the role of Mater in mammalian embryonic development.

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Mutations in *SDHC* cause autosomal dominant paraganglioma, type 3

Nonchromaffin paragangliomas (PGLs) are usually benign, neural-crest–derived, slow-growing tumours of parasympathetic ganglia. Between 10% and 50% of cases are familial and are transmitted as autosomal dominant traits with incomplete and age-dependent penetrance[1,2].

In most hereditary cases, the trait is transmitted through affected fathers but not through affected mothers, suggesting maternal imprinting (inactivation) of the disease gene. Hereditary paraganglioma is genetically heterogeneous and three loci, *PGL1* (refs 3,4), *PGL2* (ref. 5) and *PGL3* (ref. 6), have been reported. Mutations of *SDHD*, encoding the small subunit of cytochrome b in mitochondrial complex II (ref. 7), underlie PGL1. This complex contains four nuclear-encoded proteins. Subunits SDHA and SDHB constitute the catalytic domains and are anchored in the inner mitochondrial membrane by subunits SDHC and SDHD.

We reasoned that mutations in different components of mitochondrial complex II might cause other types of paraganglioma, and set out to analyse *SDHC*, *SDHA* and *SDHB* in patients from a family with the non-maternally imprinted paraganglioma type 3 (PGL3; ref. 6).

Members of the family with PGL3 are shown (Fig. 1*a*). We analysed *SDHC* in both affected and unaffected family members at both the cDNA and the genomic level. We first synthesized cDNA by RT–PCR from lymphoblastoid cell lines of patients. Sequencing the entire cDNA of 510 bp (accession number D49737) did not reveal a mutation. Because a potentially mutated transcript might not be